SECRETION OF GASES AGAINST HIGH PRESSURES IN THE SWIMBLADDER OF DEEP SEA FISHES.

II. THE RETE MIRABILE [1]

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The anatomical structure in the swimbladder of physoclist fishes associated with the gas secretion is the so-called red gland. This consists of a glandular, often button-like, structure exposed to the lumen of the swimbladder. The gland is provided with blood circulation through a so-called *rete mirabile* which consists of intermingled, parallel, unbranched, afferent and efferent capillaries. In deep sea fishes these may be more than one cm. long. In the outside layers of the swimbladder these capillaries unite to form the respective arteries and veins (Fig. 1). The structure as it varies in different fishes has been describd by Woodland (1911).

The essential feature from a functional standpoint seems to be that the *rete* represents a counter current exchange mechanism where the ingoing blood is brought into intimate diffusion contact with the outflowing blood (Müller, 1840; Woodland, 1911; Haldane, 1922; Hall, 1924; Jacobs, 1930; compare Scholander and van Dam, 1954). In principle the situation can be presented as in Figure 4.

It is obvious that to maintain a gradient of 100 atmospheres or more, the millimeter-thick swimbladder wall must be very impermeable to cut down diffusion losses. This seems to be accomplished by incorporating in the wall various kinds of solids (crystals and or fibers). In the long-nosed eel the swimbladder is strikingly silvery.

At 100 atmospheres' O_2 pressure, one volume blood would dissolve four volumes oxygen. It is therefore clear that the blood flow leaving the swimbladder would cause an impossibly great loss of oxygen unless the tension in the blood were somehow reduced before it left the bladder wall.

The problem is similar to that facing a whale moving in ice water. How can it maintain body temperature, in spite of having the big flukes circulated? This is accomplished by having the fluke arteries completely surrounded by veins, so that the arterial heat is transferred to the veins and thus remains within the bulk of the body. This very general principle of heat exchange was described by Bazett *et al.* (1948) as applicable to the *venae commitantes* in man.

Let Figure 4, I represent a counter current heat exchanger where 0° water is flowing in. It is heated to 10° at the loop by some source and returns in intimate contact with the ingoing tubes. The heat exchange will then result in the establishment of a linear temperature gradient in the tubes, as pictured (Fig. 4, II). The temperature of the leaving water would depend upon the efficiency of the heat

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exchanger, and might be one degree. If the effluent water were run out separately, isolated from the influent water, the temperature would have been 10°. Hence a ten-fold saving of heat was accomplished by the counter current exchange mechanism. If the temperature of the source of heat is fixed, it should be noted that the loop could only approach this temperature, but could not reach or exceed it.

The same situation would also obtain in regard to a diffusion exchange system in which instead of temperature we deal with gas tensions in a liquid.

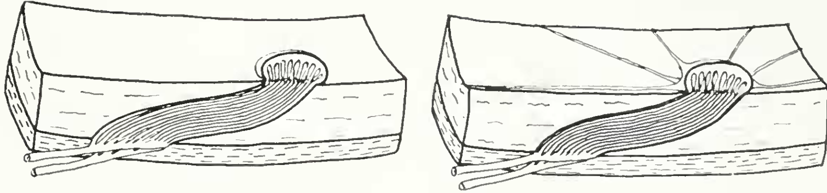

FIGURE 1. Schematic drawing of capillary *rete* and gland in relation to the swimbladder wall, which is represented with exaggerated thickness. To the left the *rete* ends in the gland (*e.g.*, in *Antimora, Coryphaenoides*); to the right blood vessels radiate from the gland over the surface of the swimbladder (*Sebastes, Synaphobranchus*).

MATERIAL

The deep sea material used in the present investigation was obtained from the Lerner Marine Laboratory, Bimini, and from cruises with the dragger *Cap'n Bill II* off the New England coast. (For particulars see Scholander and van Dam, 1954.)

HISTOLOGICAL STRUCTURE OF THE RETE

Sections of the *rete* from the long-nosed eel and the rosefish are given in Figure 2 (A-F). The *rete* of the rosefish is similar to that found in the common eel described by Krogh (1919). By differential injection he showed that the afferent smaller capillaries surround the larger efferent venous capillaries much in the same manner as is seen in the rosefish (D). In the ratfishes (C) these small capillaries surrounding the bigger ones are thick-walled and clearly arterial. In the long-nosed eel (A) both afferent and efferent capillaries tend to be squarish, about 13 $\times$ 13 μ. The walls between the capillaries average 1.5 μ. Most of the capillaries containing red cells are surrounded on all four sides by empty capillaries. It is believed from this that one *rete* was empty and that the *retes* are fitted together like the squares on a checkerboard.

The checkerboard arrangement is the geometrical arrangement which gives the maximal diffusion exchange between the afferent and efferent capillaries, and it is remarkable that we find it in our deepest fish. The only other solution to the topological problem of making four polygons (black or white) meet at one point in such a way that black always borders white is realized in the hexagonal star pattern found in the *rete* of the rosefish (D).

It would seem that the checkerboard pattern might have developed through a non-staggered columnar arrangement of the primary vessels (Fig. 3a), whereas the star pattern might have developed from a staggered pattern of the primary

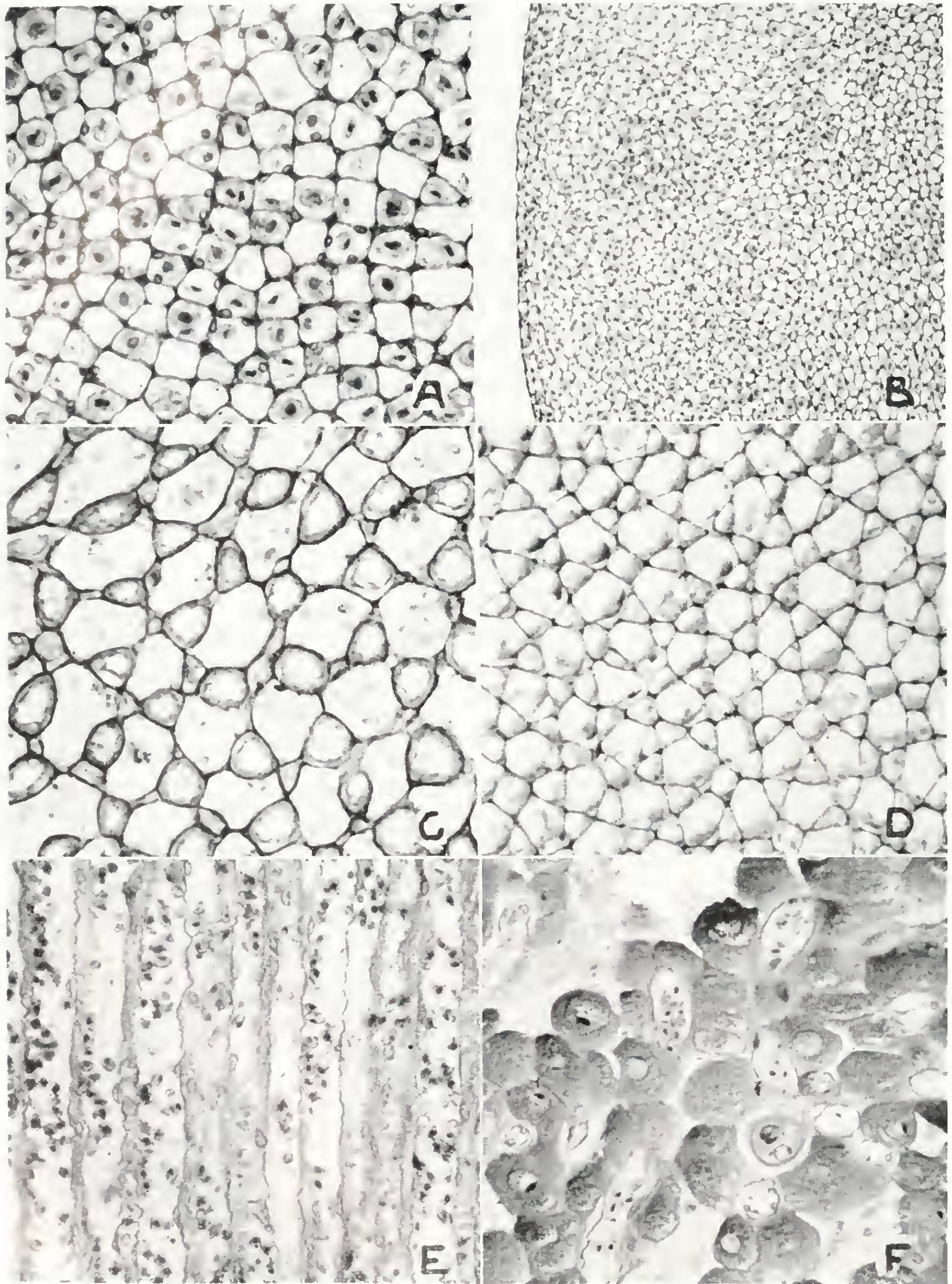

FIGURE 2. Sections through *rete* in deep sea fishes. A: *Synaphobranchus*, cross section × 300. Each polygon (square) is a single capillary. B: Same as A, × 100. C: *Coryphaenoides*, cross section × 300. D: *Sebastes*, cross section × 300. E: *Coryphaenoides*, longitudinal section × 300. Many red cells in capillaries. F: *Coryphaenoides*. Glandular epithelium from

vessels (Fig. 3b). In both cases the arterial capillaries may have developed in the interstices between the veins.

In many *retes* the loops of the glandular buttons arise from a vascular plexus located at the base of the buttons. This must naturally be so in all cases where the arterial *rete* capillaries outnumber the venous capillaries, as in the common eel, or in the rose- and ratfishes.

The length of the *rete* capillaries varies somewhat with the species, and increases generally with the depth. Measurements are given in Table 1.

Figure 2F shows the surface layers of the gland "button," with capillaries surrounded by the thick glandular epithelium.

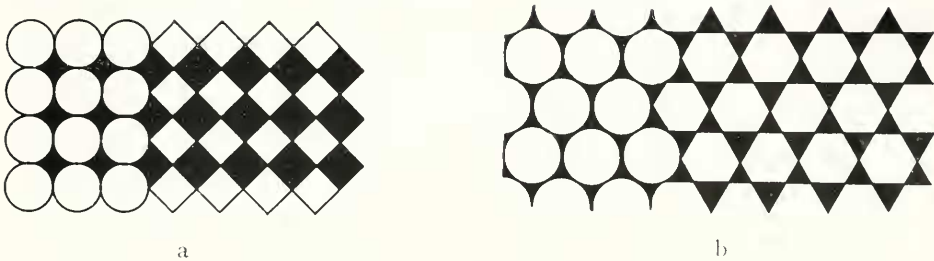

FIGURE 3. a. Possible development of the checkerboard pattern of the *rete* in *Synaphobranchus,* the primary capillaries (veins) in non-staggered rows with the arterial capillaries in the interstices. b. Possible development of *rete* in rosefish, the primary capillaries (venous) in staggered rows with the arterial capillaries in the interstices, resulting in the hexagonal star pattern.

DIFFUSION CHARACTERISTICS

From the structure and dimensions of a *rete* such as in the long-nosed eel, we may estimate what diffusion characteristics a given *rete* may have.

Let us take as unit a rete 1 cm.^{2} in cross section and 1 cm. long with capillary characteristics of the deep sea eel. The capillaries average 13 μ each side, making the circumference 52 μ. Length is 1 cm. and hence the surface area of each capillary tube is 0.0052 cm.^{2} Due to the checkered arrangement all of this surface is available for diffusion. The wall between two capillaries averages 1.5 μ. There are about 500,000 capillaries per cm.^{2} Using a diffusion constant of 150 mm.^{3} μ/cm.^{2}/min./atm. (Krogh, 1919, p. 195), we find that the diffusion across the length of this one capillary is then 0.52 mm.^{3}/min. atm. per capillary. There are 250,000 pairs of capillaries in this *rete*, and hence the total diffusion, Q, is 130 cc./min./atm.

SYMBOLS AND BASIC EQUATIONS

It is of interest to formulate a few relations pertaining to such a counter current system in order to be able to estimate more closely what a fish *rete* might be able to do.

"button," × 225. Several capillaries are seen buried among the large glandular cells. (Staining: A, B: Gomori's chrome alum-hematoxylin and phloxine, Bouin. C: Periodic acid-Schiff, Bouin. D: Periodic acid-Schiff, 80% alcohol. E: Hematoxylin and phloxine, Bouin. F: Gomori's chrome alum-hematoxylin and eosine, Bouin.) Sections and photographs by courtesy of Dept. Anatomy, Harvard Medical School.

TABLE I

Size of fish versus length of rete mirabile

Species	Depth of catch (m.)	Length	
		Fish (cm.)	*Rete* (mm.)
Rosefish	600	46	7–9
Rosefish	600	45	8–9
Rosefish	600	32	7–8
Rosefish	600	32	7–10
Rosefish	600	32	8–10
Blue hake	900	38	7–8
Blue hake	900	37	9–10
Blue hake	900	17	9
Blue hake	900	10.5	9
Common ratfish	600	34	11–13
Common ratfish	600	28	8–9
Common ratfish	600	18	8
Common ratfish	600	16	7–8

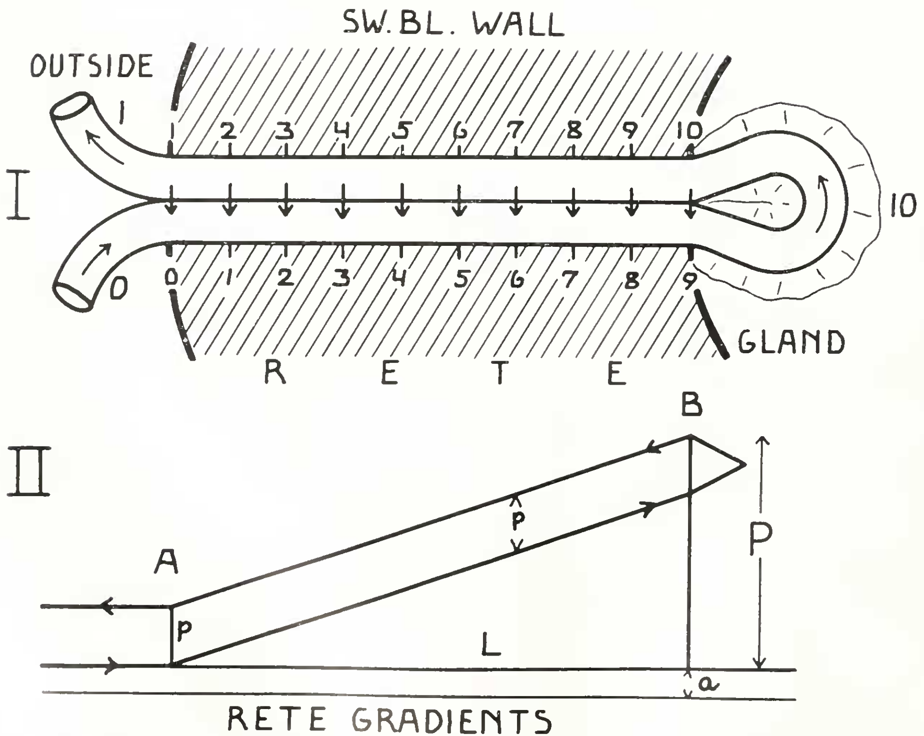

FIGURE 4. Schematic presentation of the counter current exchange principle in the swimbladder. I. Afferent and efferent capillaries. The numbers illustrate the linear gradient set up from the inside to the outside of the structure. The arrows signify the constant trans-*rete* diffusion gradient. II. Graphic representation of the gradients. L is length of the *rete;* p is trans-*rete* gradient; P is gas tension in the swimbladder; a is gas tension of inflowing blood.

Hargitay and Kuhn (1951) have developed an extensive physical treatment of this principle in relation to its probable applicability to the kidney function. Their "hairpin counter current multiplier system" is the loop of Henle. The primary concentration difference which is augmented by the system is discussed on the assumption of a semi-permeable membrane separating the afferent from the efferent flow. The difference in hydrostatic pressure due to flow presses water through the membrane and results in the primary concentration difference.

In the fish *rete* we are dealing with a different situation, inasmuch as it may be assumed that the capillaries are extremely permeable to lipid-soluble substances, especially oxygen and carbon dioxide (Pappenheimer, Renkin and Borrero, 1951), very much more so than to water. Hence it is possible to discuss the diffusion exchange in the *rete mirabile* on much simpler terms, namely, relative to what happens to gases or solutes which are simply added to the blood. The treatment is presented, realizing that it is grossly oversimplified, and at the same time it is a pleasure to credit Hargitay and Kuhn for their introduction and interpretation of the hairpin multiplier system in biology.

The following symbols will be used in the subsequent discussion:

Length of rete	= L cm.
O_2 pressure in blood entering afferent *rete*	= a atm.
Trans-*rete* gradient (O_2 tension gradient between afferent and efferent *rete*)	= p atm.
O_2 equilibrium pressure in swimbladder	= P + a atm.
O_2 pressure in blood leaving efferent *rete* (A)	= p + a atm.
Blood flow	= V cc./min.
Trans-*rete* diffusion	= Q cc./min./atm./cm.
Solubility coefficient for oxygen in blood	= α cc./cc./atm.
O_2 secreted from blood	= D cc./cc.
O_2 loss by respiration and diffusion	= R cc./min.

Based on the present fragmentary knowledge it seems possible that the oxygen can be secreted in two different ways: (a) molecular oxygen is directly split off from some compound in the blood, (b) oxygen in some form is transported to the glandular cells and stored there as a compound ready for intermittent use. These two possibilities will be dealt with separately.

a. *Release of oxygen directly from the blood.* It is here assumed that some constituent, at the present unidentified, is capable of dissociating off free oxygen in the blood against very high oxygen tensions. As blood cannot be stored in the gland to any significant degree, the release of oxygen stands in direct time relation to the blood flow. The fraction of the oxygen which is dissociated off and which stays dissociated off is called D and is without any connotation as to what is the nature of the responsible compound.

The diffusion characteristics of the *rete* may now be formulated in two basic equations (Fig. 4 II).

$$P\alpha V = pQ + p\alpha V \quad (1)$$

$$DV = p\alpha V + R \quad (2)$$

Equation 1 states that the amount of physically dissolved oxygen entering the effluent *rete* per minute (at B, Fig. 4) equals a fraction diffusing across the *rete* plus a fraction leaving the effluent *rete* (A). Equation 2 states that the amount of bound oxygen to be secreted, which enters at A, equals the amount of physically dissolved oxygen leaving at A plus an amount of oxygen lost from the swimbladder by other avenues (respiration, diffusion loss, etc.).

(i) *Quantitative evaluation of the rete function.* The oxygen secretion will first be dealt with without considering the term R. In equation 1 the term $p\alpha V$ is negligibly small compared to $P\alpha V$ and can be omitted. We then get the following relation:

$$\left.\begin{aligned} P\alpha V &= pQ \\ DV &= p\alpha V \end{aligned}\right\} \quad (3)$$

and therefore

$$P = \frac{DQ}{\alpha^2 V} \quad (4)$$

The equilibrium pressure P is consequently inversely proportional to the blood flow and directly proportional to the amount (D) of dissociated oxygen per cc. blood. P is also proportional to Q and hence to the length of the *rete*. This is borne out by the observation that for a given depth and for a given species both small and large individuals had the same length of *rete*, although the cross sections varied markedly with the size (Table I). One might make an analogy between length of *rete* and voltage, between cross section of *rete* and current (amps.).

We shall consider the *rete* equations in relation to a hypothetical but plausible deep sea fish with the following constants:

Weight	1000 g.
Swimbladder volume	50 cc.
Oxygen consumption, 8° C.	1 cc./min.
Respiratory need of swimbladder and other losses = R	0.01 cc./min.
Oxygen secreted from blood = D	0.05 cc./cc.
Oxygen solubility coefficient = α, 8° C.	0.04 cc./cc.
Trans-*rete* diffusion = Q	130 cc./min./atm.

Q varies greatly from species to species and can be given great latitude.

Let us assume a blood flow (V) through the *rete* capillaries of 1 cc. per minute and that 0.05 cc. O_2 per cc. blood (= D) is dissociated off by action of the gland. The oxygen secretion would then amount to only 50 mm.^{3}/min. or 5% of the total oxygen consumption. According to equation 4 the equilibrium pressure would then be no less than 4060 atmospheres. Obviously, therefore, if there are no losses and if the dissociation is not pressure-sensitive, the potential pressures are enormous, and would increase with lowering of the blood flow.

It is necessary to introduce a term (R) expressing the loss of oxygen by such factors as the respiratory need of the gland and the lining of the swimbladder, diffusion loss, and oxygen used for compression work.

The equations would then be:

$$\left.\begin{aligned} P &= p\frac{Q}{\alpha V} \\ DV &= p\alpha V + R \end{aligned}\right\}$$

or

$$P = \frac{(DV - R)Q}{\alpha^2 V^2} \qquad (5)$$

It is expedient at this point to consider what rates of oxygen deposition might be pertinent.

Bohr (1905) found that cod could fill its swimbladder with oxygen in about one day. Akita (1936) found the same rate for *Monacanthus*. Copeland (1952) found that *Fundulus* would need about two days, if it were able to maintain full rate for so long. If the cod and *Monacanthus* were to fill their bladders at 100 atmospheres' pressure it would take 100 days to do it. Such a drastic thing is hardly relevant for a deep sea fish. It may, however, have to compensate for a buoyancy change of, let us say, 10%, by moving from 900 meters' depth down to 1000 meters. This would require 10 days of secretion. The quantitative development of the *rete* varies greatly from species to species. Suppose that our fish can do twice as well as the cod. It would then need five days to accomplish the 10% buoyancy change and would secrete at a rate of 100 cc. oxygen of one atmosphere a day, or approximately 70 $mm.^3$/min. This would represent 7% of the total metabolic rate of the fish. Let us assume that the respiratory and diffusion loss (R) adds to it 10 $mm.^3$/min.[2] This would then give a total release of 80 $mm.^3$/min., which would require that 1.6 cc. blood per minute enter the *rete*. The potential pressure developed using these figures in equation 5 would come out as 2220 atmospheres.

When the necessary amount of gas has been deposited to accomplish the buoyancy change the fish must shut down the deposition to a simple maintenance level. This may very possibly go via a reduction of R, which may drop to, let us say, 5 $mm.^3$/min. This, according to equation 5, would make the blood flow (V) very slightly more than 0.1 cc./min., *i.e.*, 16 times less than at full secretion rate. During activity the blood vessels of the gland dilate markedly (Hall, 1924; Fänge, 1953). A less economical way of maintaining constant swimbladder volume at 100 atmospheres (or of reducing the volume) would be to draw some blood off through the non-*rete* system, like the capillaries of the "oval." Then 67 $mm.^3$/min. of O_2 would have to be run off from the swimbladder, which would require that 4 $mm.^3$ blood per minute be bled through the oval.

(ii) *Amount of oxygen secreted per cc. blood at steady state.* By considering the maximal rate at which oxygen can be deposited in the swimbladder one may calculate D, *i.e.*, the amount of dissociable oxygen carried in by the blood.

If, again, 80 $mm.^3$/min. is taken as the maximum rate of secretion and if the

[2] The work of compressing one mol gas from 0.2 to 100 atmospheres at 8° is equal to $1.99 \times T \times 2.3 \log (P_1/P_2)$ Cals. = 3.5 Cals. The caloric equivalent of one liter O_2 equals 4.8 Cal. or 104 Cal. per mol. Hence for each part of oxygen secreted into the swimbladder a minimum of 3% goes to the work of compression, or 2.1 $mm.^3$ out of the above 10 $mm.^3$ which constitute R.

blood flow (V) through the *rete* is 2 cc./mm., the blood would have to unload 40 $mm.^3$ oxygen per cc. blood, or more or less what the hemoglobin could do if dissociated.

The amount of physically dissolved oxygen in the blood is no more than 0.04 $\times$ 0.2 = 0.008 vol. % and therefore cannot be the source of a steady-state secretion, even if the solubility coefficient in the effluent blood were drastically lowered by some unknown process. The tension difference (p) between affluent and effluent blood could only be a few millimeters Hg.

b. *Secretion of oxygen from the gland cells.* Considering that we have failed to identify hemoglobin or any other compound in the blood as the direct source for the secreted oxygen, we must consider the possibility of a cellular secretion, where the oxygen is ultimately transported to the glandular cells via some compound in the blood. This compound, or a derivative thereof, might be stored in the epithelial cells for intermittent use. Non-steady-state events are conspicuously associated with the secretion. Glycogen is thus stored in large quantities by the gland cells if secretion is inhibited. When the gland is secreting, the glycogen is being used, and secretion cannot go on when the store of glycogen is depleted (Copeland, 1952; Fänge, 1953). If a breakdown of a stored compound is what determines the maximal rate of oxygen secretion, then the amount (D) of oxygen transported to the gland per cc. blood can be much smaller than would be necessary if the oxygen is released directly from the blood. From the information available it is impossible to decide between cellular secretion of oxygen and the release of molecular oxygen directly from the blood.

Conclusions. The discussion of the *rete* may be summarized in the following statements. The structure of the *rete* is such as to provide for an extremely efficient counter current diffusion exchange mechanism between inflowing and outflowing blood. This makes possible a steep tension gradient within the *rete* so that tension loss through the circulation becomes very low. Quantitative considerations of the diffusion efficiency of the *rete* make it clear that the limiting equilibrium pressure theoretically could reach tremendous values, provided the dissociation pressure of the underlying chemical or physical reaction is even higher. If oxygen cannot be chemically stored in the gland it must enter through the blood concomitantly with the secretion. This would require an amount of oxygen per cc. blood larger than that physically dissolved in the arterial blood. The nature of the responsible compound is not known.

The Role of Lactic Acid and CO_2

We have seen that thanks to the multiplication principle of the *rete,* small primary concentration effects can build up to considerable pressures, limited ultimately by the pressure characteristics of the primary chemical or physical dissociation. It was shown that the Root effect or Bohr effect at pH 6 was nullified already at 50 atmospheres in some fishes which live and secrete oxygen at much greater depths, and therefore it could not be the primary pressure reaction. Whether stronger lactic acid and lower pH could do it we do not know. It seems difficult *in vitro* to go much further without irreversible damage to the blood (Scholander and van Dam, 1954).

As regards CO_2 it was likewise shown that in some fishes 5 and 10% CO_2 would

be inadequate to dissociate off oxygen at the required pressures. The question that arises is: Could the CO_2 tension be higher than this, and if so what would be the pressure characteristics of the Bohr or Root effect?

The CO_2 content of the aerated blood of several deep sea species is given in Table II. If a small amount of lactic acid were added to the blood by the gland the CO_2 pressure would build up in the *rete* and the swimbladder, and the approximate tension gradients can be calculated, inasmuch as the diffusion constant can be taken as about 25 times that of oxygen (Krogh, 1919), and the solubility coefficient is about 1.0, or again 25 times that of oxygen. This would make $\underset{\sim}{Q}$ of equation 1 for $CO_2 = 25 \times 130 = 3250$ cc./min./atm.

If the CO_2 effect were responsible for the oxygen secretion in the wreckfish we would expect to find in the swimbladder at all times of steady-state a CO_2 tension higher than 10% of an atmosphere. A wreckfish caught at 640 meters had 0.22% CO_2 or 14% of an atmosphere. Six black groupers had from 6.6—13% of an atmosphere CO_2. In contrast to this 27 specimens of surface fishes, comprising 6

TABLE II

*Carbon dioxide content of blood**

Species	Depth of catch (m.)	Number of specimens	CO_2 (vol. %)	
			Range	Mean
A. Undiluted whole blood, aerated at 10° C.				
Black grouper	333–375	4	4.8–6.3	5.5
Alphestes	410	1	—	7.0
Wreckfish	640	2	6.8–8.0	7.4
B. Whole blood plus lactic acid 0.17 *M* (10:1), aerated at 10° C.				
Black grouper	333–375	2	1.4–1.5	1.45
Alphestes	410	1		1.9

* Method of Scholander, Flemister, and Irving (1947), modified.

species, had a CO_2 tension averaging only 0.7%. It has been shown by Jacobs (1934) and von Ledebur (1937) in 10 species of physoclist fishes, which were induced to secretion by puncture, that on the average the CO_2 tension in the swimbladder rose by only 1.4—3.1% while the O_2 rose to 80–90%. In some of the species the CO_2 did not rise at all. A *Serranus cabrilla* with the oval obliterated accumulated no more than 3% CO_2 during the process of secretion. The relatively high CO_2 tensions in deep sea fishes may hence very likely be artifacts, as CO_2 diffuses so fast. If, nevertheless, we assume that our deep sea fish were to require as much as 0.5 atm. CO_2 in the swimbladder [3] to dissociate off the oxygen, and if we assume this Root effect to be reversible when the CO_2 pressure decreases, then we can estimate the rate at which such deposition could take place.

If P = 0.5 atm., $\underset{\sim}{Q} = 3250$ cc./min., $\alpha\, CO_2$ is 1.0, and at a high rate of secretion V = 1 cc./min., then according to equation 3

$$p = 0.12 \text{ mm. Hg}$$

[3] Tautog blood begins to hemolyze at one atmosphere CO_2.

In other words the P_{CO_2} of the "venous" blood leaving the *rete* would be only 0.12 mm. Hg higher than that of the "arterial" blood entering the *rete*. Now the oxygen *tension* of the leaving blood is always slightly higher than the tension of the entering blood. The leaving hemoglobin would hence be nearly, if not completely, arterialized. If under these circumstances any oxygen at all were deposited it would be at an infinitesimally small rate.

If the leaving CO_2 tension were to be high enough to effect a half-saturation of the hemoglobin (which is necessary for the secretion rate), one would expect at least around 10 mm. Hg CO_2 tension in the leaving blood. This would, however, require some 43 atmospheres' CO_2 tension in the swimbladder, which of course is absurd.

We see therefore that unless CO_2 at tensions higher than we tested produces some *irreversible* oxygen-splitting from the hemoglobin, we cannot accept the CO_2 effect as producing the oxygen secretion.

Koch (1934) considered that if the elevated CO_2 tension passed onto the arterial side of the *rete*, the O_2 would increase there and O_2 would diffuse across to the venous side, which would seem like a short circuit with regard to the O_2. In order to avoid this he thought that buffer might diffuse across from the arterial side to the venous, due to hydrostatic forces. If this were to happen it would at best mean a lower P (CO_2) on the venous side, hence an even earlier recombination of oxygen with the hemoglobin before the blood left the *rete*, and hence no secretion.

According to either of the two theories (CO_2 and lactic acid) the oxygen is liberated directly from the blood and hence *in* the *rete*. This obviously wastes part of the *rete* as a diffusion barrier, and the oxygen must also diffuse from the *rete* into the swimbladder. Here we encounter another seemingly backwards situation, inasmuch as in many fishes the capillary loops in the gland are covered with a deep layer of epithelial cells (Fig. 2F), which must hinder very substantially the gas diffusion into the swimbladder. The loops are also usually very much fewer than the *rete* capillaries. In order to get an efficient gas exchange we would expect rather a great many almost naked capillaries, such as in the lungs.

The anatomical arrangement seems to fit a cellular gas secretion much better. This would leave the whole *rete* as a simple counter current diffusion barrier against oxygen loss, and the secreting cells covering the capillary loops would aid the *rete* as a diffusion barrier, rather than counteract it.

There are grave difficulties in seeing the Bohr or Root effects as the cause of the secretion, and at the present time it seems indicated that many observed events associated with the secretion of oxygen, such as the occasional large rise in CO_2 (Jacobs, 1930; von Ledebur, 1937; Rostorfer, 1942), the acid formation (Hall, 1924), the glycogen disappearance (Copeland, 1952; Fänge, 1953), carbonic anhydrase activity (Leiner, 1938, 1940; Copeland, 1952; Fänge, 1953), potential glycolytic activity, lactic acid formation (Strittmatter, Ball and Cooper, 1952; Fänge, 1953) must be fitted into the secretion picture in some other way.

If dissociation of oxyhemoglobin still is to be considered as the direct cause of the pressure build-up, it must be brought about by a diffusible x-substance (from the gland cells), which would dissociate off oxygen against much higher pressure than does lactic acid. This x-substance would either have to leave with the blood and must then be quite indiffusible through the *rete* capillaries, in contrast to the

gland capillaries, or if easily diffusible it would have to effect an irreversible reaction, such as methemoglobin formation, to be repaired outside the gland. Such a substance and such diffusion conditions are purely hypothetical, although perhaps possible.

There are at the present time unresolved obstacles in regarding the Root or Bohr effect as the secretion mechanism. It has not been proved that hemoglobin is involved, and it would be premature to disregard the possibility of a cellular oxygen secretion.

Conclusions. In several deep sea fishes the blood acidified with lactic acid to pH 6 or lower arterializes fully at a much lower oxygen pressure than that existing in the swimbladder. Hence lactic acid via the Root effect cannot be the cause of oxygen secretion. If the CO_2 effect were to be the cause of the secretion, CO_2 would have to leave the gland at a pressure of at least 5–10 mm. Hg. This would

TABLE III

Nitrogen content of the swimbladder gas in deep sea fishes

Species	Depth of catch (m.)	Number of specimens	% N_2	Mean partial pressure of N_2 (atm.)
Black grouper	320–400	6	12.5–16.8	5.4
Alphestes	410	1	18.5	7.6
Wreckfish	640	1	11.0	7.0
Rosefish	660	2	20.0	13.2
Long-finned hake	1300	5	3.0–3.7	4.4
Blue Hake	1240	4	6.0–7.6	8.0
Round-nosed ratfish	1100	3	9.4–10.0	10.8
	1200–1240	3	8.4–10.3	11.6
Common ratfish (*Macrourus bairdii*)	1240	4	7.2–7.8	9.3
Long-nosed eel	1200	1	7.6	9.1

require a CO_2 pressure in the swimbladder of 20–40 atmospheres, which is out of the question. If hemoglobin is directly involved in the oxygen secretion the mechanism by which it is dissociated is unknown. One is led to consider seriously the possibility of a cellular oxygen secretion.

SECRETION OF INERT GASES

It has been shown that the nitrogen tension found in the swimbladder of marine fishes is practically always higher than in the sea water. In each species the nitrogen is deposited as a relatively constant proportion of the oxygen, but the fraction varies from species to species. It usually lies between 2 and 15%. In deep sea fishes the N_2 tension can therefore reach as much as 15–20 atmospheres. New data from greater depths confirm these conclusions, Table III and Figure 5. It does not seem possible to explain the high nitrogen as an artifact (Scholander and van Dam, 1953).

The whitefish (Hüfner, 1892) and several more species of freshwater physo-

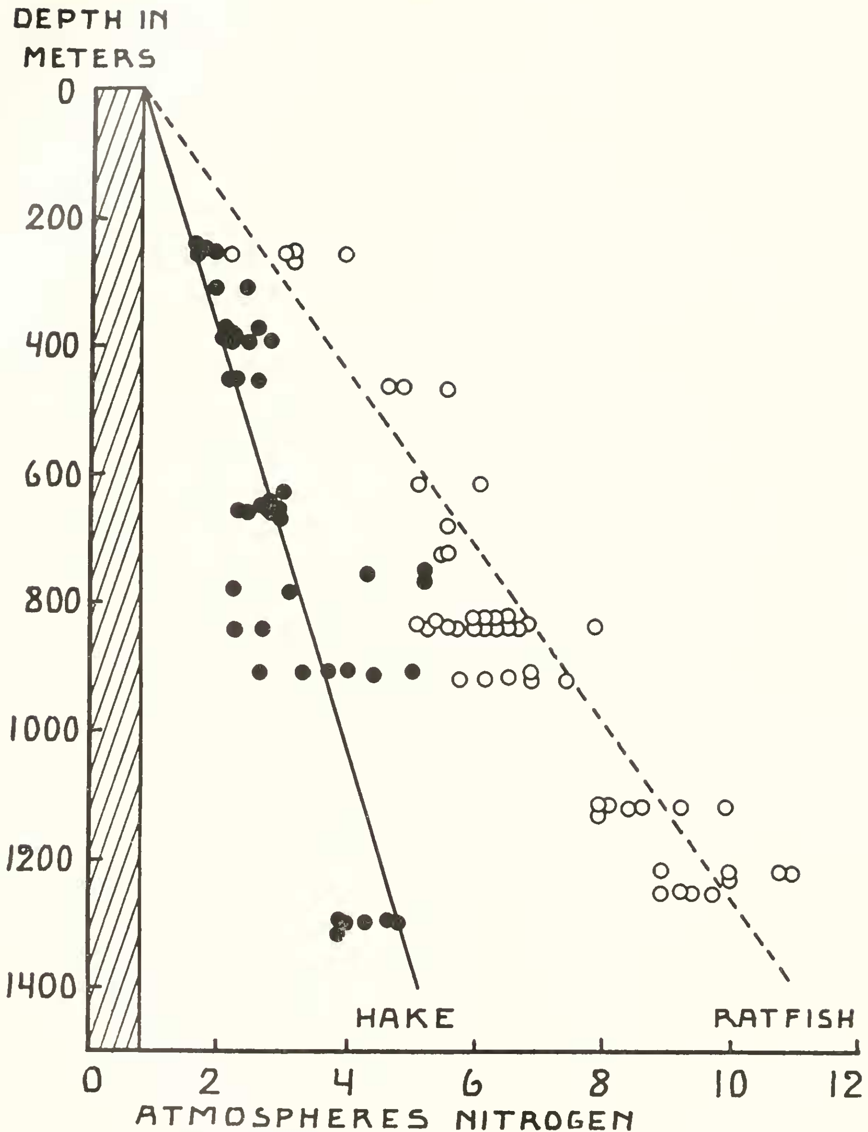

FIGURE 5. Relation of nitrogen tension in swimbladder to depth for the long-finned hake (*Urophycis chesteri*) and common ratfish (*Macrourus bairdii*).

stomes (Saunders, 1953) are able to deposit "pure" nitrogen against some 6–8 atm. pressure.

The argon-nitrogen ratio in the swimbladder. An important clue as to how basically nitrogen enters the swimbladder can be obtained by comparing the argon-nitrogen ratio in the swimbladder gas to the ratio in the air, as pointed out by Koch (1934). He considered, and rightly so, that the fact that the A/N_2 ratio found by

Schloesing and Richard (1896) was near the atmospheric ratio indicated that the nitrogen could not have been chemically secreted.

Some of our deep sea samples have been analyzed for argon. This was made possible through the courtesy of Dr. A. O. C. Nier and of Mr. Bailey Donnally, who kindly made the analyses for us on the mass spectrometer. The results will be seen in Table IV and are in favor of a physical deposition of nitrogen. If nitrogen were secreted chemically, the argon/N_2 ratio would be decreased in the given fishes between 6 and 20 times, *i.e.*, it would have been found to be between 0.2 and 0.06, instead of 1.18. Although there are substantial deviations from the air ratio they are certainly not of the order which would suggest nitrogen secretion by chemical means. Considering the fact that the argon may be expected to have

TABLE IV

Argon-nitrogen ratio in the swimbladder gas of deep sea fishes

Species	Depth of catch (atm.)	% (N_2 + A)	N_2 + A atm.	$\frac{100A}{N_2}$
Air	1	80	0.8	1.18
Blue hake		6.2		1.16
Blue hake		6.0		1.22
Common ratfish	80	7.2	5.8	1.00
Common ratfish	80	7.6	6.1	1.61
Common ratfish	80	7.4	5.9	1.08
Rosefish	50	20.0	10.0	0.94
Rosefish	50	20.0	10.0	0.87
Long-nosed eel*	167			1.94
Moray eel*	9			1.85
Air*	1			1.18

* Schloesing and Richard, 1896. Long-nosed eel, *Synaphobranchus pinnatus*; Moray eel, *Muraena helena*.

twice the diffusion coefficient of nitrogen, one would expect the argon fraction to tend to be low rather than high (Scholander and van Dam, 1953).

There are unfortunately no data available on the argon/N_2 ratio in the whitefish, nor on the presence of organic gases, which would appear as nitrogen.

THEORIES FOR SECRETION OF INERT GASES

Two theories have been offered to explain the high nitrogen tensions.

a. *The bubble theory.* Powers *et al.* (1932) assumed that the Root effect would result in the formation of bubbles and that these when entering the swimbladder would transport nitrogen in from the surroundings. If the Root effect were responsible, the highest possible local pressure increase would be in the blood, and the bubbles would form there, and as Koch (1934) pointed out, it is hard to see how they would get out of the blood. Haldane (1922) pointed out that "bends" in human divers occur only if the pressure is diminished by more than 50% at a time. The highest increase in the plasma which dissociation of 9 vol. % oxygen

could give is 3 atmospheres. Operating with blood equilibrated at 100 atmospheres' oxygen pressure in our cuvette, bubbles never formed at sudden drops of pressure, even ten times 3 atmospheres. Hence in deep sea fishes gas bubbles cannot very well form in the capillaries of the gland or of the *rete*. Experimentally, gas bubbles have not been observed to form within living cells, even after decompression from very high gas pressures (Harvey *et al.*, 1944). In shallow fishes, however, gas bubbles were seen to form in the mucus covering the gland when the gland was stimulated to activity (Fänge, 1953).

b. *Secretion by change in the solubility coefficient.* The second theory, proposed by Koch (1934), seems to offer a possible mechanism for the increase in nitrogen and inert gases. He considered the possibility that by some means the blood in the swimbladder vessels would have a lower solubility coefficient for gases than it does outside the swimbladder. This could come about by an increase in the temperature of the active gland, or by added solutes.

Any metabolic heat gradient would certainly be very small, as the swimbladder wall has no aspect of being a thermal insulator. Due to the extraordinry exchange capacity of the *rete* there would be, if any, only an infinitesimal difference in the temperature of blood entering and leaving the *rete*. Hence they would have the same nitrogen capacity and N_2 deposition could then not occur (equation 6).

If the solubility were depressed by the addition of some solute entering the blood from the gland, the nitrogen tension would slightly increase on the venous side. If this situation persisted so that the blood would leave the *rete* with a decreased solubility coefficient the following relation would obtain, using the symbols given above and essentially equations 1 and 2:

$$\left.\begin{aligned} P\alpha_2 V &= pQ \\ a\alpha_1 V &= (a + p)\alpha_2 V \end{aligned}\right\} \qquad (6)$$

$$P = \frac{a(\alpha_1 - \alpha_2)Q}{\alpha_2^2 V} \qquad (7)$$

The numerical values for the constants are: $a = 0.8$ atm.; $\alpha_1 = 0.0202$ cc./cc./atm.; $\alpha = 0.0200$ cc./cc./atm.; $V = 1$ cc./min. Q is directly proportional to the diffusion constant which for N_2 is about half that of oxygen. Q is therefore for nitrogen 65 cc./cm./cm.^{2}/min./atm.

If we have a 1% decrease in solubility at the exit of the *rete* the equilibrium pressure P would be 26 atmospheres. Theoretically, therefore, one could get enough pressure built up. It is obvious that the rate of N_2 deposition by this means would be exceedingly slow, since each cubic centimeter of blood would only transport in 0.16 mm.^{3} N_2/min.

A striking thing about the nitrogen tension in the swimbladders of our marine fishes is that in most of them it increases linearly with the depth, as if the nitrogen were produced as a constant percentage of the oxygen secretion. This observed phenomenon ($P_{N_2}/P_{O_2} = 2$–15%, and independent of pressure) could only be derived from the equations 5 and 7 if R were either proportional to the pressure or if it were constant, neither of which assumption seems likely to be true. There is a further difficulty with the proposed theory where an x-substance produces the lowering of the solubility coefficient, in that if the substance were diffusible it would

be likely to concentrate enormously in the inner part of the *rete*. And if it were not diffusible how would it have entered the blood stream in the first place? Another serious difficulty is that physostomes like the whitefish evidently lack a typical *rete*.

Conclusions. The nitrogen-to-argon ratio in the swimbladder of deep sea fishes was found to be approximately that of the sea water and atmosphere, confirming what was found earlier by Schloesing and Richard (1896). In these fishes, therefore, the nitrogen has not been chemically secreted.

The theory of Koch (1934) that nitrogen and argon might be secreted into the swimbladder by a decreased solubility coefficient in the effluent blood of the *rete* has been evaluated quantitatively. Although high enough equilibration pressures of nitrogen could be achieved by a small change in the solubility coefficient, the constant ratio N_2/O_2 at all depths, as actually found, cannot readily be explained by this theory. The theory may not apply to physostomes, like the whitefish, which seem to lack a typical *rete*.

During this work I have received many suggestions from stimulating discussions with Dr. A. C. Redfield and Dr. L. van Dam at the Woods Hole Oceanographic Institution. I am much obliged to Dr. A. O. C. Nier and to Mr. Bailey Donnally at the Physics Department of the University of Minnesota for mass spectrographic analyses of the argon/nitrogen ratio in the swimbladder gases. Dr. George Wislocki, Department of Anatomy, Harvard Medical School, has generously given his help and advice with regard to the histological material. This long-preserved material was most skillfully prepared by Mrs. Edith Herman and photographed by Mr. Leo Talbert of the same department. I am much indebted to Dr. C. M. Breder, Jr., of the American Museum of Natural History, for arranging our work at the Lerner Marine Laboratory, and I wish to thank Mr. Michael Lerner and the staff of the Lerner Marine Laboratory for all the help they extended to us. Mr. W. C. Schroeder of the Woods Hole Oceanographic Institution gave us the best of facilities on board the dragger *Cap'n Bill II*, and for much assistance in the laboratory and with the manuscript thanks are due to Dr. L. van Dam and Mrs. Susan I. Scholander.

Summary

1. The structure and dimensions are given for the *rete mirabile*. It is interpreted as a counter current diffusion exchange mechanism between the afferent and efferent *rete*.

2. This diffusion exchange has been quantitatively evaluated for O_2, CO_2, and N_2. The arrangement makes possible the maintenance of a steep tension gradient within the *rete*, so that the oxygen loss from the leaving blood can be extremely low.

3. Quantitative evaluation of the efficiency of the *rete* diffusion makes it clear that the limiting equilibrium pressure in the swimbladder could be extremely high, and that the limiting factor lies mainly in the dissociation pressure of the chemical or physical reaction which ultimately splits off the oxygen.

4. The nature of the responsible compound and reaction is unknown. The anatomical arrangement of the glandular structure and the *rete* is suggestive of a

cellular secretion of oxygen rather than of a mechanism that splits off oxygen in the blood.

5. Data on the deposition of nitrogen against high pressures which were previously found in deep sea fish have been confirmed and extended. The argon-to-nitrogen ratio in the swimbladder gas suggests that the nitrogen has entered the swimbladder via some physical mechanism. The possibility that this is brought about by a lowering of the nitrogen solubility coefficient in the efferent *rete* is discussed.

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